

Factors Contributing to the Acute and Subchronic Adverse Respiratory Effects of Machining Fluid Aerosols in Guinea Pigs

Terry Gordon¹ and Karen Galdanes

New York University School of Medicine, Tuxedo, New York 10987

Received May 29, 1998; accepted January 19, 1999

Several physical, chemical, and microbial factors are potential contributors to the adverse pulmonary effects associated with occupational exposure to machining fluid aerosols. The present study examined the relative toxicity of 3 major classes of machining fluids (soluble, semi-synthetic, and synthetic) as well as that of unused (fresh) versus used (grab samples taken from manufacturing sites) machining fluids. Pulmonary function and changes in cellular and biochemical indices in bronchoalveolar lavage fluid were examined during and 24 h after exposure, respectively. Statistically significant differences in toxicity were observed in guinea pigs exposed for 3 h to respirable aerosols of unused machining fluids (semi-synthetic > soluble >> synthetic). In addition, greater toxicity was observed in animals exposed to used, machining fluid aerosols compared to unused fluids. Moreover, within the used machining fluid types, significantly greater adverse effects were observed in animals exposed to poorly maintained fluids (i.e., heavy microbial contamination) versus well-maintained fluids. Changes in biochemical and cellular parameters in bronchoalveolar lavage fluid occurred after a single exposure to 5 mg/m³ of the poorly maintained used machining fluid aerosols. Changes in inflammation but not LDH and protein were observed in animals repeatedly exposed to semi-synthetic machining fluid aerosols. A statistically significant increase in lavage fluid neutrophils was observed in guinea pigs exposed to 5 mg/m³ used, semi-synthetic machining fluid aerosols for 4 weeks. In separate experiments, physicochemical properties of unused machining fluids were found to contribute to the production of adverse effects. Adjustment of the alkaline and hypotonic nature of the unused semi-synthetic machining fluid to isotonicity and pH 7 significantly reduced adverse effects. Together, these findings strongly suggest that multiple factors contribute to the adverse respiratory effects associated with occupational exposure to machining fluid aerosols.

Key Words: alkaline aerosols; toxicity; endotoxin; pulmonary injury; occupational.

Epidemiologic studies and industrial hygiene surveys have clearly demonstrated that occupational exposure to machining fluid aerosols in metal grinding and cutting operations is associated with tumors in various organs and adverse respiratory

effects (Eisen *et al.*, 1992; Kennedy *et al.*, 1989; Tolbert *et al.*, 1992). A 5% or greater cross-shift decrease in forced-expiratory volume at 1 s occurred in 23% of automotive workers exposed to machining fluid aerosols (versus 9.5% in the control group of assembly workers), demonstrating that these aerosols can produce acute, reversible airway obstruction (Kennedy *et al.*, 1989). In addition, some oil mist-exposed workers acquire asthma (Robertson *et al.*, 1988; Rosenman *et al.*, 1995), while others develop bronchitis, cough, or increased sputum production, following long-term exposure to machining fluid aerosols (Jarvholm, 1982; Oxhoj *et al.*, 1982). Thus, both acute and chronic respiratory effects are associated with occupational exposure to machining fluid aerosols.

Despite the strong evidence that machining fluid aerosols can produce adverse respiratory effects, identification of the machining fluid components responsible for these adverse effects has been difficult. In general, work-related respiratory effects appear to occur regardless of the type of machining fluid used. Metal working fluids used during machining processes contain many potentially toxic or irritating agents (Table 1). A portion of these components or factors are intentionally added to the machining fluid to achieve optimal lubricating and antimicrobial (e.g., formaldehyde-releasing agents) characteristics and as antifoaming agents. The machining fluids are also maintained at an alkaline pH to inhibit corrosion and microbial growth.

Identification of factors contributing to the production of adverse respiratory effects is further complicated by the different types of machining fluids in use. These fluid types include soluble, semi-synthetic, synthetic, and straight fluids (Table 1), although the latter is used less frequently in modern operations. Animal toxicity studies have attempted to identify potential agents responsible for the differences in toxicity. Schaper and Detwiler (1991) reported significant differences in the relative irritancy of various machining fluid types in mice. This laboratory has previously found that endotoxin contamination is a major contributor to the changes in pulmonary function and the lung inflammation and injury observed in guinea pigs exposed to a soluble machining fluid aerosol (Gordon, 1992). Similarly, (Thorne *et al.* (1996) have reported inflammatory changes in the lungs of mice and guinea pigs

¹ To whom correspondence should be addressed at Department of Environmental Medicine, NYU School of Medicine, 57 Old Forge Rd., Tuxedo, NY 10987. Fax: (914) 351-5472. E-mail: gordont@charlotte.med.nyu.edu.

TABLE 1
Chemical Constituents of Machining Fluids

Machining fluid	Components	Max %
Soluble:	Mineral oil	50
	Ethanolamine	10
	Nonylphenoxy-polyethoxyethanol	10
	Triethanolamine	10
Semi-synthetic:	Mineral oil	30
	Ethanolamine	10
	Nonylphenoxy-polyethoxyethanol	10
	Triethanolamine	10
Synthetic:	Organic acid	10
	Ethanolamine	10
	Caprylic acid	10
	Triethanolamine	10
	Isononanoic acid	10

exposed to used machining fluid aerosols. One purpose of the present study was to determine whether machining fluid types differ in their ability to produce toxic pulmonary effects. Guinea pigs were challenged with a single 3-h exposure to aerosols of unused, soluble, semi-synthetic, and synthetic machining fluids, and examined for changes in pulmonary function and cellular and biochemical indices of injury and inflammation in lavage fluid. We also investigated whether the induction of adverse respiratory effects was related to: (1) the alkaline pH and hypotonicity of machining fluid aerosols; and (2) the presence of microbial contamination in used machining

fluids sampled from actual worksites. Finally, we examined whether the adverse respiratory effects observed after a single exposure were exacerbated by subchronic exposure to machining fluid aerosols.

MATERIALS AND METHODS

Animals. Viral-antibody-free, male, Hartley outbred guinea pigs (410 to 551g; Charles River Breeding Laboratories, Kingston, NY) were fed standard guinea-pig chow (Purina Lab Chow) and water *ad libitum*. They were housed in polycarbonate cages with corncob bedding located within a positive pressure, HEPA-filtered isolator. The animals were kept on a 12-h light/dark cycle starting at 6 A.M. and the rooms maintained at $20 \pm 2^\circ\text{C}$ and $50\% \pm 10\%$ relative humidity. The study was conducted in accordance with guidelines set by the NYU School of Medicine's animal use committee. Guinea pigs were chosen as the test animal because of their demonstrated sensitivity to inhaled machining fluids and endotoxin (Gordon *et al.*, 1991, 1992; Thorne *et al.*, 1996).

Single exposures. Groups of animals were exposed for 3 h to nebulized water (n , 5) or 2 concentrations of 3 different types of machining fluids: soluble, semi-synthetic, and synthetic (n , 6/group) (Table 2). For each machining fluid type, a group of animals was exposed to the following aerosols: (1) the unused machining fluid (unused, neat machining oil diluted to 5% (by volume) in pyrogen-free water [Baxter Laboratories, Deerfield, IL]); (2) clean machining fluid (a worksite sample of used machining fluid with minimal microbial contamination); or (3) dirty machining fluid (a worksite sample of used machining fluid with significant microbial contamination). The target concentrations for each type of machining fluid aerosol were 5 and 50 mg/m^3 . Animals were exposed to the lower target concentration of 5 mg/m^3 only if significant changes were observed in 2 or more lavage parameters or pulmonary function as a result of exposure to 50 mg/m^3 .

To examine the role of pH and osmolarity on the response to machining fluid

TABLE 2
Exposure Atmosphere Parameters

Exposure	n	Machining fluid concentration (mg/m^3)	Endotoxin concentration ($\mu\text{g}/\text{m}^3$)	Mass median aerodynamic diameter (μm)	Geometric standard deviation
Water	5	NA	BDL	NA	
Soluble-unused-5 mg/m^3	6	4.5	BDL	1.0	1.80
Soluble-unused-50 mg/m^3	6	52.8	0.05	1.2	1.93
Soluble-clean-5 mg/m^3	6	5.2	BDL	1.0	1.72
Soluble-clean-50 mg/m^3	6	52.0	0.01	1.3	1.85
Soluble-dirty-5 mg/m^3	6	5.1	0.04	1.0	1.75
Soluble-dirty-50 mg/m^3	6	49.1	0.45	1.4	1.76
Semi-synthetic-unused-5 mg/m^3	6	6.3	BDL	0.8	1.71
Semi-synthetic-unused-50 mg/m^3	6	51.9	0.01	0.8	1.71
Semi-synthetic-clean-5 mg/m^3	6	5.2	BDL	1.1	1.69
Semi-synthetic-clean-50 mg/m^3	6	45.6	BDL	1.5	1.83
Semi-synthetic-dirty-5 mg/m^3	6	5.6	0.13	0.9	1.75
Semi-synthetic-dirty-50 mg/m^3	6	47.5	0.55	1.2	2.00
Synthetic-unused-5 mg/m^3	NT	NT	NT	NT	NT
Synthetic-unused-50 mg/m^3	6	53.5	0.13	1.1	1.95
Synthetic-clean-5 mg/m^3	NT	NT	NT	NT	NT
Synthetic-clean-50 mg/m^3	6	49.3	0.01	1.2	1.79
Synthetic-dirty-5 mg/m^3	6	7.2	0.27	0.8	2.04
Synthetic-dirty-50 mg/m^3	6	48.9	1.92	1.1	2.09

Note. NA, not applicable; NT, not tested; BDL, below detection limit of 0.003 $\mu\text{g}/\text{m}^3$.

aerosols, groups of animals were exposed to samples of the unused, semi-synthetic machining fluid (50 mg/m^3) and the used, dirty semi-synthetic machining fluid (10 mg/m^3), which were adjusted to pH 7 (with 1 N sulfuric acid), and/or 300 milliosmoles (with pyrogen-free 0.9% sodium chloride). The machining fluids were a gift of Cincinnati Milacron (P. Ronald Yust, Cincinnati, OH). Machining fluid samples were kept at 4°C during storage to inhibit microbial growth. The chemical and microbiological characteristics of the 3 machining fluid types are listed in Tables 1–3. Data on the metal content (atomic absorption spectroscopy), pH, and bacteria and mold titers (culturable colonies/ml) were provided by Cincinnati Milacron. Sani-Check BF dipsticks (Biosan Laboratories, Warren, MI) were used to determine culturable bacteria and mold titers.

During exposure, animals were restrained in a head-out restrainer (Agrawal, 1981). Double O-rings, which circumscribed the restrainers, ensured that an airtight seal was maintained during the head-only exposure. The dynamic exposure chamber (56 liters) was constructed of acrylic plastic and was operated under slightly negative pressure ($<0.1 \text{ cm H}_2\text{O}$). Aerosols were generated with a Babington-type nebulizer (Solosphere, Airlife Inc., Modesto, CA) driven by medical-grade breathing air. To produce the different target concentrations of machining fluid aerosols, the delivery pressure of air to the nebulizer was varied between 3 and 14 psi. The output of the nebulizer (4.42 liters/min at 9 psi) was diluted with 15 to 43 liters/min of HEPA- and activated charcoal-filtered air prior to entering the exposure chamber. Airborne machining fluid concentrations were determined gravimetrically by taking samples of the chamber atmosphere in the breathing zone of the animals, at 1.07 liters/min during each exposure, using 47-mm diameter polyvinyl chloride filters ($0.8 \mu\text{m}$, Omega Specialty Instrument Co., Chelmsford, MA). Filters were weighed with a microbalance (Cahn Instruments Inc., Cerritos, CA) before and after sampling. Post-sampling filter weights were recorded after the microbalance had stabilized (approximately 2 min) to ensure that low vapor pressure components of the machining fluid, such as water, contributed minimally to the gravimetric analyses. Particle-size distribution measurements were performed gravimetrically, using a Mercer mini-cascade impactor (Intox Products, Albuquerque, NM) with glass substrates. The particle size distributions for the various machining fluids are listed in Table 2. Additional cellulose acetate or glass fiber filter samples were collected, extracted, and analyzed for endotoxin levels using sterile techniques.

After sampling, filter media were immediately placed in sterile, pyrogen-free glass containers and stored at 4°C until extracted. To extract endotoxin from the filters, 30 ml of sterile, pyrogen-free water (Baxter) was added to each sample. The samples were placed in a 68°C water bath (Millner *et al.*, 1988) for 30 min. The extracts were decanted and placed at 4°C to cool and stored until analysis. Samples were assayed the same day as extraction. Test tubes, pipets, pipet tips, filters, water, and microplates were routinely analyzed to ensure that there was no prior pyrogen contamination. Previous work established that cellulose acetate collection medium was superior to other filter media for extraction of endotoxin from soluble-type machining fluids (Gordon *et al.*, 1992). Similar preliminary analyses established that cellulose acetate and glass fiber filters were suitable for endotoxin extraction and analysis of semi-synthetic and synthetic/soluble machining fluids, respectively. Endotoxin concentrations were quantitated with a *Limulus* amoebocyte lysate assay (QC1000, Whittaker Bioproducts, Walkersville, MD) using a spectrophotometric microplate method. The assay results were compared to a standard NBS traceable endotoxin and expressed in terms of endotoxin units (EU) or nanograms (10 EU's was assumed to equal 1 ng). The airborne endotoxin concentrations were expressed in $\mu\text{g/m}^3$.

Pulmonary function. In each single exposure group, specific airway conductance (SGaw) was measured in awake and spontaneously-breathing animals at 5 min intervals for 15–20 min prior to exposure and at 60 min intervals during exposure. As previously described (Thompson *et al.*, 1987), SGaw was determined in a whole body, constant volume plethysmograph based on the design of Agrawal (1981). Briefly, animals were restrained by the posterior chamber of the Agrawal plethysmograph during the head-only exposures and were removed for approximately 2 min for each measurement of SGaw. SGaw

was calculated from an estimate of airway driving pressure and air flow measured at the snout with a pneumotachograph (model #0, Fleisch Instruments, Lausanne, Suisse). Air-flow and box-pressure signals were calibrated daily and simultaneously delivered to an oscilloscope. SGaw was calculated from the slope of the rising limb of the resulting loop and corrected for pressure and temperature as described by Agrawal (1981).

Biochemical studies. At 24 h post-exposure, guinea pigs were euthanized with an overdose of 175 mg/kg of pentobarbital and exsanguinated by transecting the inferior vena cava. Each guinea pig was tracheostomized, cannulated, and lavaged twice with 10 ml of sterile, pyrogen-free, phosphate-buffered saline (Gibco-BRL, Gaithersburg, MD). Total cell counts in the lavage fluid were done with a hemocytometer. Lavage fluid was centrifuged at $400 \times g$ for 10 min and aliquots of the supernatant analyzed for protein content and lactate dehydrogenase (LDH) activity. Protein was determined on diluted aliquots (stored at -20°C) of lavage fluid by a commercially available microassay kit (BioRad, Berkeley CA). The samples were assayed in duplicate and compared to a standard curve prepared with bovine serum albumin (Sigma Chemical Co., St. Louis, MO). LDH activity was determined in duplicate with a commercially available kit (Sigma) on the same day on which the lavage procedure was performed. LDH activity was expressed as Berger-Broida units (BB units) where one BB unit is equivalent to 0.48 International Units of activity. Cell-differential slides (100 cells/slide per animal) were prepared by cytocentrifugation and stained with Giemsa (Harleco, Gibbstown, NJ) dissolved in 0.2 M TRIS-maleate buffer and adjusted to pH 5.5.

Repeated exposures. Groups of animals (n , 10/group) were exposed to nebulized water, 5 mg/m^3 unused semi-synthetic machining fluid, and 1 or 5 mg/m^3 used semi-synthetic machining fluid for 3 h/day, 5 days/week, for 4 weeks. SGaw was examined before study initiation, at 2 weeks, and immediately after the 4-week exposure period. At these time points, nonspecific airway responsiveness to acetylcholine was determined using methodology previously described (Gordon *et al.*, 1988). At the end of the exposure period, guinea pigs were euthanized, their lungs lavaged (as described above), and biochemical and cellular markers of injury and inflammation examined (at 24 h; n , 6/group or 72 h; n , 4/group), after termination of exposures.

Statistical analyses. In the single exposure experiments, SGaw values during exposure were normalized to pre-exposure baseline values for each animal. For each exposure concentration, the effect of exposure on SGaw was then analyzed by a 2-factor analysis of variance (ANOVA) with independent factors of fluid type and condition (unused, clean, or dirty) considered. When indicated, a Student Newman Keul's test was used to compare normalized SGaw values between all groups. Biochemical and cell-count data were also analyzed by a 2-factor ANOVA followed by a Student Newman Keul's test, when applicable. Comparison of multiple exposure groups to the control (water)-group values was performed with a 2-way Dunnett's t test. In the repeated exposure experiment, all parameters were analyzed by a 1-factor ANOVA and, when indicated, a *post hoc* Dunnett's t -test to compare the machining fluid-exposure groups to the water-control group. Levels of $p \leq 0.05$ were accepted as evidence of significant group differences.

RESULTS

Single Exposures

The actual measured machining fluid concentrations for the target concentrations of 5 and 50 mg/m^3 are listed in Table 3. Because pulmonary effects were not observed in the groups of animals exposed to 50 mg/m^3 of either the unused or clean, used, synthetic machining fluid aerosols, the effect of a lower concentration was not tested. The particle-size distribution was similar for all the machining fluid types at each level of exposure (Table 3). The mass median aerodynamic diameter ranged from 0.8 to $1.5 \mu\text{m}$ and the geometric standard devia-

TABLE 3
Chemical and Microbial Characteristics

Fluid type	Class	Metal concentrations (PPM)				pH	Osmolarity (milliosmoles)	Bacterial titer ^a	TcMold titer
		Cd	Cu	Pb	Zn				
Soluble	Unused	0.0	0.0	0.0	0.14	7.0	69	ND	ND
Soluble	Clean	0.16	0.62	3.0	0.07	8.8	109	6.5E+04	1.0E+03
Soluble	Dirty	0.16	0.74	2.3	0.92	7.8	94	1.0E+07	BDL
Semi-synthetic	Unused	0.0	4.0	0.0	7.4	9.0	81	ND	ND
Semi-synthetic	Clean	0.3	0.67	0.36	0.96	8.8	111	1.0E+03	0.0E+00
Semi-synthetic	Dirty	0.0	0.0	0.11	0.0	9.0	142	1.0E+06	0.0E+00
Synthetic	Unused	0.0	0.0	0.0	0.0	9.5	147	ND	ND
Synthetic	Clean	0.0	0.21	0.0	0.24	9	192	1.0E+06	0.0E+00
Synthetic	Dirty	0.0	0.46	0.0	0.52	8.5	149	1.0E+06	0.0E+00

Note. ND, not determined; NA, not applicable; BDL, below detection limit.

^a Titer is defined as the number of colony-producing bacteria or molds/ml.

tion ranged from 1.7 to 2.1. The concentration of metals was similar among the machining fluids with the exception of a greater level of Cu and Zn in the unused, semi-synthetic machining fluid (Table 1).

The 2-factor ANOVA demonstrated that the independent factor of machining fluid type had a significant effect on the outcome of response to 50 mg/m³ of the machining fluid aerosols ($p < 0.0001$). Significant differences in lavage fluid protein were observed among the groups of guinea pigs exposed to the 3 types of unused, machining fluid aerosols. Also, in comparison to control (water) animals, exposure to 50 mg/m³ of the unused, semi-synthetic and soluble machining fluids produced significant increases in LDH and protein in lavage fluid, whereas a significant increase in only LDH

occurred in animals exposed to the unused, synthetic machining fluid (Table 4). Overall, the changes in lavage fluid parameters in the unused, semi-synthetic machining fluid animals were greater than the response of animals to the other 2 types of unused machining fluids. At 5 mg/m³, no statistically significant changes in lavage parameters were noted in animals exposed to any of the unused machining fluid aerosols (Table 4).

The condition of the machining fluid also significantly affected the outcome of response. With one exception (lavage fluid protein after exposure to 50 mg/m³ of the semi-synthetic machining fluid), exposure to the dirty, used machining fluid aerosols produced greater changes in lavage fluid parameters than did the corresponding concentration of the clean used

TABLE 4
Effect of Machining Fluid Exposure on Lavage Fluid Parameters*

Exposure	50 mg/m ³			5 mg/m ³		
	LDH	Protein	PMNs	LDH	Protein	PMNs
Water	25.2 ± 5.8	325.1 ± 26.1	0.1 ± 0.0	25.2 ± 5.8	325.1 ± 26.1	0.1 ± 0.0
Soluble-unused	87.5 ± 3.0 ^a	691.9 ± 49.7 ^{a,b}	2.3 ± 0.4 ^c	25.2 ± 5.0	198.7 ± 12.8	0.1 ± 0.1
Soluble clean	284.0 ± 14.8 ^a	947.3 ± 65.2 ^a	51.7 ± 3.1 ^a	16.8 ± 3.8	209.3 ± 11.9	0.1 ± 0.0
Soluble dirty	297.3 ± 8.9 ^a	1348.3 ± 156.7 ^{a,c}	106.8 ± 6.6 ^{a,c}	52.8 ± 7.4	479.2 ± 20.1	1.9 ± 0.8
Semi-synthetic-unused	94.8 ± 11.0 ^a	851.5 ± 37.8 ^{a,b}	7.0 ± 0.7 ^c	27.4 ± 2.4	238.2 ± 14.1	0.1 ± 0.0
Semi-synthetic-clean	184.7 ± 30.5 ^a	1460.6 ± 205.3 ^a	52.8 ± 5.0 ^a	58.3 ± 6.3	424.7 ± 25.3	0.1 ± 0.1
Semi-synthetic-dirty	285.9 ± 11.8 ^{a,c}	952.2 ± 123.7 ^{a,c}	102.2 ± 17.5 ^{a,c}	112.7 ± 38.7 ^{a,c}	519.8 ± 18.6	6.3 ± 1.0
Synthetic-unused	69.1 ± 5.1 ^a	247.0 ± 17.2 ^b	0.3 ± 0.1	NT	NT	NT
Synthetic-clean	32.2 ± 3.8	209.6 ± 19.5	0.1 ± 0.0	NT	NT	NT
Synthetic-dirty	327.3 ± 0.8 ^{a,c}	1780.5 ± 185.6 ^{a,c}	71.0 ± 7.4 ^{a,c}	245.7 ± 25.9 ^a	1033.7 ± 136.5	32.9 ± 7.9 ^a

Note. NT, not tested.

^a Statistically different ($p < 0.05$) from the control (water) group values.

^b Statistically different ($p < 0.05$) from the other unused machining fluids at 50 mg/m³—not analyzed at 5 mg/m³.

^c Statistically different ($p < 0.05$) from the corresponding clean used machining fluid.

* Data are presented as mean ± SE. LDH, BB units/ml; protein, μg/ml; PMNs, total PMNs × 10⁶.

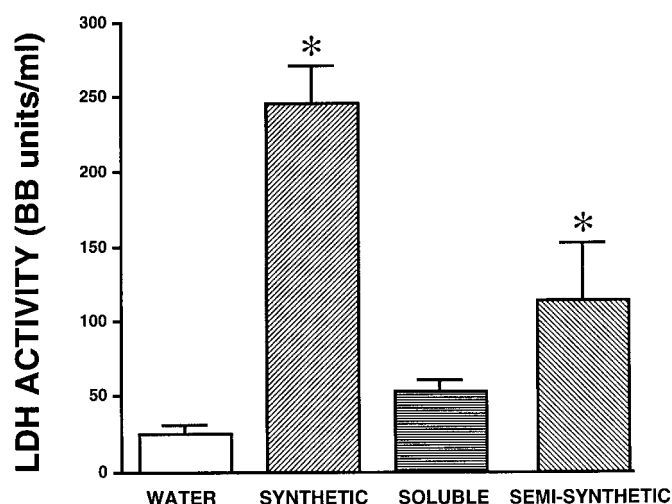


FIG. 1. Effect of exposure to 5 mg/m³ dirty used machining fluid aerosols on LDH activity (mean + SE) in the bronchoalveolar lavage fluid of guinea pigs. Berger-Broida units/ml are abbreviated as BB units/ml; *indicates that the value was statistically different from control (water) animal values ($p < 0.05$).

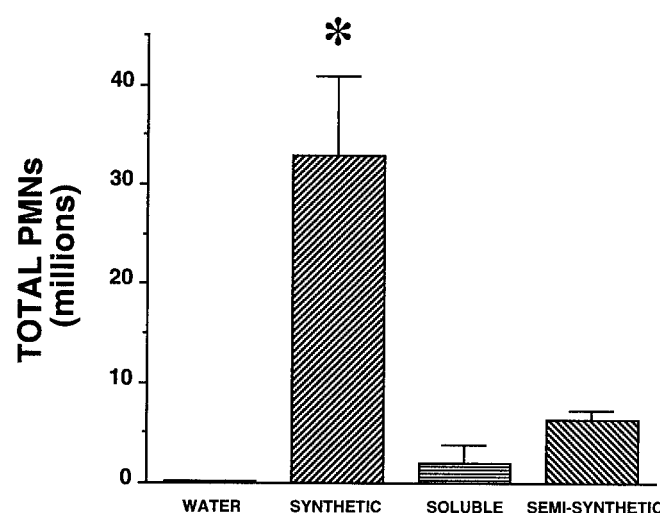


FIG. 2. Effect of exposure to 5 mg/m³ used dirty machining fluid aerosols on the number of PMNs (mean + SE) in the lavage fluid of guinea pigs; *indicates that the value was statistically different from control (water) animal values ($p < 0.05$).

machining fluid aerosols (Table 4). Notably, the well maintained clean synthetic machining fluid produced no significant increases in lavage fluid parameters.

At the 5 mg/m³ exposure level, significant effects were observed only in animals exposed to the dirty machining fluid aerosols. Compared to control animal values, significantly increased levels of LDH activity (Fig. 1) and protein were found in the lavage fluid of animals exposed to 5 mg/m³ of the dirty, synthetic and semi-synthetic machining fluid aerosols. In addition, a significant increase in PMNs occurred in animals exposed to the dirty, used synthetic machining fluid aerosols at this exposure level (Fig. 2).

Both pH and osmolarity had significant effects on the response of guinea pigs to unused, semi-synthetic machining fluid aerosols. Adjustment of the alkaline and hypotonic fluid to pH 7 and 300 milliosmoles, alone or in combination, significantly reduced the injury (increased protein and LDH) and inflammation (total PMNs) resulting from a single 3 h exposure to 50 mg/m³ of the unused, semi-synthetic machining fluid aerosols (Fig. 3). Manipulation of the pH and osmolarity, however, did not blunt the statistically significant increases in PMNs, LDH, and protein observed in guinea pigs exposed to 10 mg/m³ of the dirty semi-synthetic machining fluid (Fig. 4).

Machining fluid exposure had no statistically significant effect on SGaw except for a 20% decrease in animals at the end of the 3-h exposure to either the 5 or 50 mg/m³ used, dirty semi-synthetic machining fluid aerosols (data not shown).

In the repeated exposure study, significant increases in lavage fluid neutrophils were observed in animals at 24 h after repeated exposure to 5 mg/m³ used, semi-synthetic machining fluid ($6.67 \times 10^4 \pm 0.95 \times 10^4$, mean \pm SE) compared to animals exposed to nebulized water ($1.10 \times 10^4 \pm 0.41 \times$

10^4). No other significant lavage fluid or pulmonary function changes were observed in these animals or in animals exposed to 1 mg/m³ used, semi-synthetic or 5 mg/m³ unused machining fluid aerosols.

DISCUSSION

A number of adverse health effects have been associated with occupational exposure to machining fluid aerosols. Identification of the machining fluid components responsible for

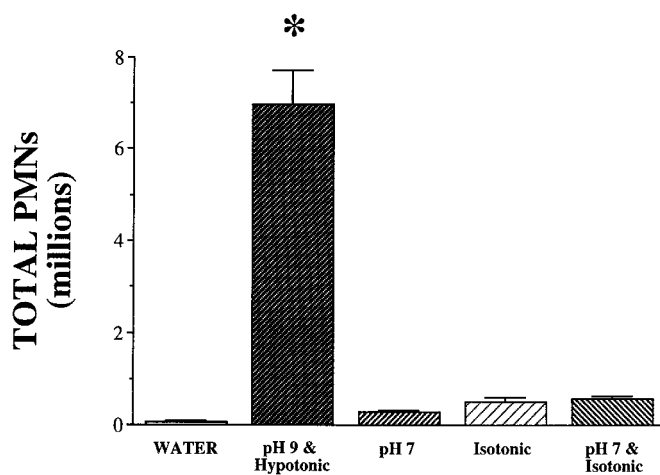


FIG. 3. The role of unused machining fluid pH and osmolarity on the number of PMNs (mean + SE) in bronchoalveolar lavage fluid. Groups of guinea pigs were exposed to aerosols of the unused semi-synthetic machining fluid (50 mg/m³), which was either untreated (i.e., pH 9 and hypotonic) or brought to pH 7 and/or 300 milliosmoles (isotonic) using sulfuric acid and sodium chloride, respectively; *indicates that the value was statistically different from control (water) animal values ($p < 0.05$).

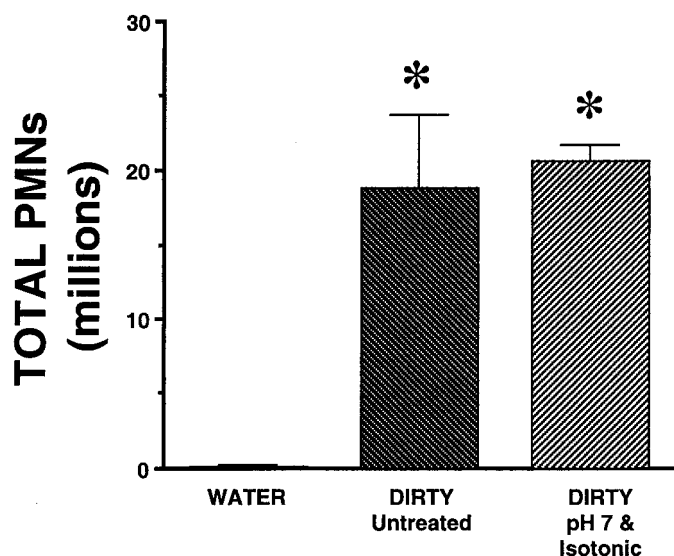


FIG. 4. The relative contribution of machining fluid pH and osmolarity to the inflammatory response of guinea pigs exposed to contaminated machining fluid aerosols (mean + SE). Groups of animals were exposed to aerosols of the used dirty semi-synthetic machining fluid (10 mg/m³), which was untreated or brought to pH 7 and 300 milliosmoles (isotonic) using sulfuric acid and sodium chloride, respectively; *indicates that the value was statistically different from control (water) animal values ($p < 0.05$).

these adverse effects has been hindered by the complex nature of the chemical constituents and contaminating microbial agents in these fluids. As a first step in determining the contribution of the chemical constituents, the present study examined the adverse functional, biochemical, and cellular effects of the 3 major fluid types currently used in machining operations. The study clearly demonstrated that fluid type had a significant impact on outcome ($p < 0.0001$). Following exposure to the unused machining fluids at the high concentration of 50 mg/m³, both the unused, semi-synthetic and soluble aerosols produced significant increases in 2 of the 3 parameters of lung injury and inflammation examined. A significant change in only lavage fluid protein was observed in the animals exposed to 50 mg/m³ of the unused, synthetic machining fluid aerosols. Although these findings may not apply across the board to the many and continually changing formulations of the 3 major machining fluid types, they point out that differences in toxicity do exist among the machining fluids and should be considered in their use and future design.

The present study also determined that for each of the 3 different machining fluid types, the used, dirty (i.e., poorly maintained) machining fluids were more toxic than the used, clean (i.e., well-maintained) or unused machining fluids. This finding confirms earlier reports (Gordon, 1992; Thorne *et al.*, 1996) and suggests that changes in the chemical constituents or microbial contamination of machining fluid contribute strongly to the production of lung injury and inflammation in this guinea pig model. As shown in Table 1, there were no major differences in the metal content, pH, and/or osmolarity of the used

machining fluids, and thus, these factors likely played a small role in the observed inter-fluid differences in toxicity. Extensive chemical analyses of the unused and used machining fluids were not performed and would be necessary to elucidate the possible contribution of other factors, such as the organic components, to the observed adverse respiratory effects.

Importantly, the increases in adverse effects produced by the used, dirty machining fluid aerosols were associated with the number of viable bacteria in the fluids, as well as the presence of endotoxin in the aerosols. Thus, as suggested by previous work (Gordon, 1992), it is likely that contaminating endotoxin or other microbial contaminants contribute to the adverse effects of poorly-maintained machining fluids. An increase in PMNs in lavage fluid, a sensitive marker of adverse effects after exposure to the low concentration of the used, dirty machining fluid aerosols, is a well-known result of endotoxin inhalation (Gordon *et al.*, 1991; Helander *et al.*, 1982). Moreover, the increase in PMNs at 24 h after the final exposure was the only significant change observed in animals repeatedly exposed to the used, dirty machining fluid aerosols over 30 days. Thus, despite the apparent development of tolerance to the acute changes in lavage fluid protein and LDH, inflammatory changes persisted. This increase in PMNs was absent in animals examined at 72 h after the final exposure, thus suggesting recovery from the 30-day exposure regimen.

The alkaline pH and the hypotonicity of the machining fluids may play a role in the adverse effects associated with inhalation of machining fluid aerosols. This study demonstrated that adjusting the pH and osmolarity to 7.0 and 300 milliosmoles, respectively, markedly inhibited the effects of exposure to 50 mg/m³ of the semi-synthetic machining fluid. The relative contribution of these physical/chemical factors, however, was apparently less than that of the contribution of the changes in machining fluids that occur during use and storage at the worksite. This was demonstrated by the lack of inhibition of observable adverse effects in animals exposed to 5 mg/m³ of the pH- and osmolarity-adjusted, used, dirty, semi-synthetic machining fluid aerosols (Fig. 4).

In conclusion, animal studies have the capability to provide important information regarding the toxicity of complex mixtures such as machining fluid aerosols (Gordon, 1992; Schaper and Detwiler, 1991). This study demonstrated inherent differences in the toxicity of the 3 major machining fluids used in machining operations today. Thus, end users may consider the potential toxicity, in addition to the functionality, of the machining fluid types in the choice of cutting and lubricating fluids for machining operations. Alternatively, machining fluid manufacturers may be able to adjust the chemical components to reduce the respiratory toxicity of machining fluids. The present study also presented evidence that a single 3-h exposure to 5 mg/m³ of unused machining fluids does not produce any observable functional, biochemical, or cellular adverse effects in an animal model. Thus, workplace exposure to well-maintained and noncontaminated machining fluid aerosols may

be without acute adverse effects. During the routine use and storage of tens of thousands of gallons of machining fluids, however, bacterial and fungal contamination does occur (Foxall-VanAken *et al.*, 1986; Mattsby-Baltzer *et al.*, 1989). The present study has demonstrated that microbial contaminants, pH, and osmolarity may each contribute to the acute, adverse effects reported for machining fluid-exposed workers (Kennedy *et al.*, 1989).

ACKNOWLEDGMENTS

The authors would like to thank P. Ronald Yust (Cincinnati Milacron, Cincinnati, OH) for assistance in obtaining and characterizing the machining fluids used in these studies and Dr. Richard Schlesinger for assistance in the statistical analyses. This work was supported by Public Health Service Grants OH03044, ES-00260 and ES-0256. TG was the recipient of a Research Career Development Award from the National Institute of Environmental Health Sciences.

REFERENCES

- Agrawal, K. P. (1981). Specific airway conductance in guinea pigs: normal values and histamine induced fall. *Respir. Physiol.* **43**, 23–30.
- Eisen, E. A., Tolbert, P. E., Monson, R. R., and Smith, T. J. (1992). Mortality studies of machining fluid exposure in the automobile industry. I. A standardized mortality ratio analysis. *Am. J. Ind. Med.* **22**, 809–824.
- Foxall-Van Aken, S., Brown, A., Jr., Young, W., Salmeen, I., McClure, T., Napier, S., Jr., and Olsen, R. H. (1986). Common components of industrial metal-working fluids as sources of carbon for bacterial growth. *Appl. Environ. Microbiol.* **51**, 1165–1169.
- Gordon, T. (1992). Acute respiratory effects of endotoxin-contaminated machining fluid aerosols in guinea pigs. *Fundam. Appl. Pharmacol.* **19**, 117–123.
- Gordon, T., Balmes, J., Fine, J. M., and Sheppard, D. (1991). Airway oedema and obstruction in guinea pigs exposed to inhaled endotoxin. *Br. J. Ind. Med.* **48**, 629–635.
- Gordon, T., Galdanes, K., and Brosseau, L. (1992). Comparison of sampling media for endotoxin-contaminated aerosols. *Appl. Occup. Env. Hyg.* **7**, 472–477.
- Gordon, T., Thompson, J. E., and Sheppard, D. (1988). Arachidonic acid metabolites do not mediate toluene diisocyanate-induced airway hyperresponsiveness in guinea pigs. *Prostaglandins* **35**, 699–706.
- Helander, I., Saxen, H., Salkinoja-Salonen, M., and Rylander, R. (1982). Pulmonary toxicity of endotoxins: Comparison of lipopolysaccharides from various bacterial species. *Infect. Imm.* **35**, 528–532.
- Jarvholm, B. (1982). Cutting oil mist and bronchitis. *Eur. J. Respir. Dis.* **63**(suppl. 118), 79–83.
- Jarvholm, B., Bake, B., Lavenius, B., Thiringer, G., and Vokmann, R. (1982). Respiratory symptoms and lung function in oil mist-exposed workers. *J. Occup. Med.* **24**, 473–479.
- Kennedy, S. M., Greaves, I. A., Kriebel, D., Eisen, E. A., Smith, T. J., and Woskie, S. R. (1989). Acute pulmonary responses among automobile workers exposed to aerosols of machining fluids. *Amer. J. Ind. Med.* **15**, 627–641.
- Mattsby-Baltzer, I., Sandin, M., Ahlstrom, B., Allenmark, S., Edebo, M., Falsen, E., Pedersen, K., Rodin, N., Thompson, R. A., and Edebo, L. (1989). Microbial growth and accumulation in industrial metal-working fluids. *Appl. Environ. Microbiol.* **55**, 2681–2689.
- Millner, P. D., Perkins, H. H., Jr, and Harrison, R. E. (1988). Methods for assessment of the endotoxic respirable dust potential of baled cotton. In *Proceedings of the 12th Cotton Dust Research Conference* (P. J. Wakelyn and R. R. Jacobs, Eds.), pp. 3–5. National Cotton Council, Memphis, TN.
- Oxhoj, H., Andreasen, H., and Henius, U. M. (1982). Respiratory symptoms and ventilatory lung function in machine-shop workers exposed to coolant-lubricants. *Eur. J. Respir. Dis.* **118**(suppl), 85–89.
- Robertson, A. S., Weir, D. C., and Burge, P. S. (1988). Occupational asthma due to oil mists. *Thorax* **43**, 200–205.
- Rosenman, K. D., Reilly, M. J., Watt, F. C., and Kalinowski, D. J. (1995). Annual report on occupational asthma in Michigan. Michigan State University Department of Medicine and Michigan Department of Public Health and Bureau of Occupational Health.
- Schaper, M., and Detwiler, K. (1991). Evaluation of the acute respiratory effects of aerosolized machining fluids in mice. *Fundam. Appl. Toxicol.* **16**, 309–319.
- Thompson, J. E., Gordon, T., Scypinski, L. A., and Sheppard, D. (1987). Tachykinins mediate toluene diisocyanate-induced airway hyperresponsiveness. *Amer. Rev. Respir. Dis.* **136**, 1118–1121.
- Thorne, P. S., DeKoster, J. A., and Subramanian, P. (1996). Pulmonary effects of machining fluids in guinea pigs and mice. *Amer. Ind. Hyg. Assoc. J.* **57**, 1168–72.
- Tolbert, P., Eisen, E., Pothier, L. J., Monson, R. R., Hallock, M. F., and Smith, T. J. (1992). Mortality studies of machining fluid exposure in the automobile industry. II. Risks associated with specific fluid types. *Scand. J. Work Environ. Health* **18**, 351–360.